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ANTIGENIC AND IMMUNOGENIC PROPERTIES OF STAPHYLOCOCCAL DELTA-TOXIN

USSR

Following is a translation of an article by Ye. V. Rusakova, Institute of Epidemiology and Microbiology imeni Gamaleya of the Academy of Medical Sciences USSR, Moscow, in the Russian-language journal Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No 12, Moscow, 1967, pages 114-118.

Staphylococcal delta-toxin is one of the exotoxins frequently produced by pathogenic staphylococci. It is a large-molecule compound of protein nature (molecular weight about 70,000-100,000), consisting of 19 amino acids (Yoshida, 1963; Hallander, 1963). Only certain properties of delta-toxin are known: the hemolytic and leukolytic activity, thermal stability, cytopathogenic effect on a tissue culture; and there exist certain data on its dermanecrotic and lethal effect. But until recently little study had been made of the problem of the antigenic and immunogenic properties of delta-toxin; these properties are very important from the standpoint of the possibility of using it for immunological prevention of staphylococcal infections.

In the literature available to us we found only two works devoted to a study of this problem. McLeod (1963) immunized rabbits with native heated and unheated delta-toxin. When the course of immunization injections was completed, the rabbits received intravenously a lethal dose of native toxin. The survival of the experimental rabbits was higher than that of the control. Kayser and Raynaud (1965) carried out immunization with partially purified preparations of delta-toxin. The authors noted a 5-6-fold increase in the neutralizing activity of the sera of rabbits immunized with delta-toxin over normal sera. On the basis of these data, the conclusion is drawn in both works that the preparations of delta-toxin studied possess antigenic properties.

The purpose of our work was to study the antigenic and immunogenic activity of staphylococcal delta-toxin. We immunized rabbits with concentrated

and partially purified preparations of delta-toxin and investigated the sera of these animals by various methods.

The immunization experiments were run on chinchilla rabbits weighing 2.5-3 kilograms. There were two patterns for subcutaneous immunization: 1) three injections of 0.25 milliliter of delta-toxin at seven-day intervals and subsequent revaccination after 40-60 days; 2) injections of 0.1 milliliter of the preparation three days in a row and after ten days the cycle of injections was repeated. Immunization was carried out with two preparations of delta-toxin: a preparation concentrated with ammonium sulfate with subsequent dialysis, and a preparation partially purified by gel-filtration on sefadex G-100. The toxin was mixed with an equal amount of Freund's adjuvant before administration to the rabbits. The sera were investigated 7, 14 and 21 days after completion of the course of vaccination injections, and 7 and 14 days after revaccination.

The presence of antibodies was determined by a number of methods: the neutralization test, the ring precipitation test, immunological precipitation, and Hoigner's reaction. The passive hemagglutination reaction and the complement fixation reaction could not be used since delta-toxin has pronounced hemolytic activity with regard to the erythrocytes of many species of animals.

In setting up the reaction of neutralization of hemolysis caused by delta-toxin, we prepared double dilutions of serum and added one milliliter of delta-toxin diluted with physiological solution to one milliliter of serum, so that the final concentration was 1 Dhm. The mixture was incubated for ten minutes at room temperature, human erythrocytes washed off three times and diluted five times were added drop-by-drop to each test tube, and then the test tubes were placed in a thermostat for one hour at 37°. The maximum dilution of serum completely neutralizing the toxin was taken as the titer of the reaction.

In the ring precipitation reaction we used whole serum; over this a layer of delta-toxin was spread, diluted 10, 50, 100 and 1,000 times with physiological solution; the mixture was incubated at 37° for one hour. The immunological precipitation reaction in gel by Oukhterloni's /transliterated from Russian/ method was set up on a slide placed in a moist chamber and left there for one day at room temperature, after which time the result of the reaction was noted.

Hoigner's reaction, suggested in 1955 for discovering the minimum amounts of antibodies with respect to medicinal preparations, is based on recording microbe precipitation between antigen and antibodies with the sensitive FEKN-57 photoelectrocolorimeter. When a transparent solution of antigen is added to the serum, its optical density steadily decreases; if the microprecipitate is formed, then this reduction is temporarily retained (plateau) or the density begins to constantly increase. In setting up the reaction, 1.7 milliliters of whole serum are placed in a glass container,

and 1.1 milliliters of toxin diluted ten times with physiological solution is added, thoroughly stirred, and the optical density is measured two minutes after standing at room temperature. The diluted toxin is added to the serum ten times; thus, the total amount of toxin added in the dilution of 1:10 amounts to one milliliter. The earlier the microprecipitate forms, the more pronounced the avidity of antibodies, and the higher the intensity of the reaction.

In this investigation we used sera taken from the rabbits on the eve of the experiment. The antigen used was a preparation of delta-toxin partially purified with gel-filtration through sefadex G-100, with a hemolytic titer of 1:80-1:160.

The usual method was used to determine the content of alpha-antitoxin in all the sera investigated (Vygodchikov, 1963).

Sera taken from nonimmunized rabbits were used as a control for all the reactions.

In investigating the sera of immunized rabbits, we first of all attempted to use the reaction of neutralization of hemolysis caused by delta-toxin, by analogy with titration of alpha-antitoxin. The results of these experiments are shown in Tables 1 and 2. The data obtained indicated that sera of immunized and nonimmunized rabbits neutralized the hemolytic activity of delta-toxin almost in the same degree; therefore, this reaction cannot be used as a basis of a judgment concerning the presence of antibodies specific to delta-toxin in the sera of the rabbits, since we know that inactivation of hemolysis caused by delta-toxin can result from the nonspecific inhibiting influence of the proteins of normal serum.

Table 1

Dkm of Delta-toxin	Sera of Immunized Rabbits		Normal Sera	
	No of Samples	Average Titer	No of Samples	Average Titer
1:20-1:80	31	1:70.4	14	1:64.7
1:160	41	1:49.1	10	1:42.8
1:320	24	1:117	8	1:87.7
Total	96		32	

We must note that it was only after multiple immunization with delta-toxin and Freund's adjuvant that we were able to observe a rather substantial content of antibodies in the sera of the experimental rabbits, and in our opinion this indicates the weak antigenic and immunogenic properties of delta-toxin.

Table 2

Dhm of Delta-toxin	Sera of Immunized Rabbits: No of Samples	Titer 1:20-1:40	Titer 1:80-1:160	Titer 1:320	Normal Sera: No of Samples	Titer 1:20-1:40	Titer 1:80-1:160	Titer 1:320
1:20-1:80	31	15	16	0	14	6	8	0
1:160	41	27	14	0	10	6	4	0
1:320	24	4	16	4	8	3	5	0
Total	96	46	46	4	32	15	17	0

Table 3

Group of Rabbits	No of Samples of Sera	No of Precipitation Lines: 0	1	2 or More (up to 5)
Immunized	129	0	21	108
Nonimmunized	38	18	15	5

On the basis of these immunological precipitation reactions in gel we made a judgment concerning the presence of the antigen-antibody complex forming between delta-toxin and the sera being investigated. The results of these reactions are given in Table 3, which makes it evident that the sera of immunized rabbits formed a larger number of precipitation lines with delta-toxin than normal sera. The precipitation lines were also more distinct and intense.

Table 4

Group of Rabbits	No of Samples of Sera	Titer of Ring Precipitation Test: 0	1:10	1:50	1:100	1:1,000
Immunized	32	0	6	4	7	15
Nonimmunized	17	4	9	2	2	0

The ring precipitation test was used to determine the quantitative content of antibodies to delta-toxin; the titer of this test was taken to be the highest dilution of toxin which formed a precipitation ring with the serum being investigated. The data obtained are given in Table 4. Substantial differences were observed between the experimental and control sera (most of the experimental sera had titers of 1:100-1:1,000, whereas the titer of control sera was equal to 1:10).

Table 5

Group of Rabbits	No of Sera	Intensity of Reaction -	±	++	+++	++++
Control	21	10	6	4	1	0
Experimental						
Immunized three months after vaccination	11	0	4	4	3	0
Seven days after revaccination	12	0	3	2	7	0
Fourteen days after revaccination	12	0	2	3	6	1
After intracutaneous administration of delta-toxin	12	0	0	2	5	5

Note: ++++ indicate a continuous rise in optical density after the third-fifth addition of toxin; +++ indicate a pronounced plateau or temporary rise in optical density with subsequent reduction in optical density, caused by dissociation of the antigen-antibody complex in the excess of antigen; ++ indicate the presence of a plateau after the fifth-eighth addition of toxin; + indicates the presence of a plateau after the eighth-ninth addition of toxin; - indicates a continuous reduction in optical density.

The results of the study of the sera with Hoigner's reaction are given in Table 5, where the experimental group is represented by 12 rabbits which receive injections of delta-toxin in the sequence indicated in the table. These data indicate the pronounced difference between the sera of nonimmunized and immunized rabbits both with respect to the number of positive reactions, and with respect to the intensity of these reactions.

Table 6

Group of Rabbits	No of Sera	Content of Alpha-Antitoxin (in AE/ml) 0.125	0.25	0.5	1
Immunized	63	40	7	13	3
Nonimmunized	60	55	1	4	0

Along with the investigations described above, we determined the content of alpha-antitoxin in the sera of immunized and control rabbits. On the basis of the data presented in Table 6, we can observe that most of the sera of both groups contained 0.125 AE per milliliter, although in the experimental group there were more sera containing from 0.25 to 1 AE per milliliter than in the control group. The reason for this is probably that during the immunization period the rabbits could have undergone latent or slight forms of staphylococcal infection resulting in a certain rise in the level of alpha-antitoxin in the blood.

In investigating normal sera (as is evident from the tables) we noted that when they were combined with delta-toxin precipitation lines formed in the gel and that they resulted in positive ring precipitation reactions (in a few titers) and in Hoigner's reaction. Moreover, normal sera neutralized the hemolytic activity of delta-toxin to a substantial degree. All of this allows us to assume the existence of normal antibodies to delta-toxin in the sera of nonimmunized animals, on the analogy with the presence of a certain level of alpha-antitoxin.

Thus, when these methods are used we can establish the presence of antigenic and immunogenic activity of preparations of staphylococcal delta-toxin.

Conclusions

1. Staphylococcal delta-toxin has antigenic and immunogenic activity, although it obviously is a relatively weak antigen.

2. Antibodies to delta-toxin discovered with Hoigner's reaction, the ring precipitation test, precipitation in gel, did not neutralize the hemolytic activity of delta-toxin, but had precipitating properties.

3. Antibodies to delta-toxin were discovered in the sera of nonimmunized rabbits.

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